FENITROTHION ACCUMULATION BY PLANTS AND INVERTEBRATES IN TWO EXPERIMENTALLY-SPRAYED STREAMS IN NEW BRUNSWICK

Submitted To

Environment New Brunswick

November 1981

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6 November 1981

Mr. K. Browne
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P.O. Box 6000
Fredericton, New Brunswick

Dear Mr. Browne:

We are pleased to present our report entitled "Fenitrothion Accumulation by Plants and Invertebrates in Two Experimentally-Sprayed Streams in New Brunswick". Fenitrothion residues were found in all sampled plants and invertebrates. In certain taxa these residues were surprisingly high and persistent. To clarify certain trends, follow-up sampling has been recommended.

We trust this report meets your requirements and would be pleased to assist in future programs.

Yours very truly,

Keith D. Phinney

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Branch Head

A. Sosiak

A.J. Sosiak
Project Biologist

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SUMMARY

Aquatic invertebrates and plants were sampled in streams before and for seven days after experimental spraying with fenitrothion. Both plants and animals were analyzed for fenitrothion, 3-methyl-4-nitrophenol, fenitro-oxon and aminofenitrothion content. A water sample collected in one stream before spraying had traces of fenitrothion. The concentration of fenitrothion in water samples from plant and invertebrate sampling sites on the two streams increased after spraying to a maximum of 4.1 and 11.3 µg/L within six hours of spray application. In both streams, the fenitrothion concentration decreased to less than 1% of the peak concentration after 24 hours.

Fenitrothion residues were found before spraying in Solenostoma sp., swamp candle (Lysimachia terrestris), manna grass (Glyceria borealis), and Draparnaldia sp., and in the invertebrates Simulium sp., Heptageniidae, Neophylax sp. and Pycnopsyche sp. All plants and animals sampled contained post-spray fenitrothion residues. Highest concentrations in this study were found in the liverwort Solenostoma sp., which had peak concentrations of 2.94 and 9.0 µg/g (dry weight) in the two streams. Relatively high concentrations of fenitrothion remained in this organism seven days after spraying. The bioaccumulation ratios for this liverwort were apparently the highest yet reported for an aquatic plant exposed to fenitrothion. Fontinalis sp., slender spikerush (Eleocharis acicularis) and manna grass had their highest measured fenitrothion residues six hours after spraying. However, residues in water-mat (Chrysosplenium americanum), Riccia sp., swamp candle, Draparnaldia sp. and all four invertebrate taxa peaked later in the sampling program. The highest fenitrothion residue detected in an invertebrate was 10.0 µg/g, measured in Simulium sp. 24 hours after spraying.

Residues in all organisms except Solenostoma sp. had decreased markedly after seven days. The degradation product 3-methyl-4-nitrophenol was found in three plant samples early in the sampling program, but not

thereafter. One post-spray Heptageniidae sample also contained 3-methyl-4-nitrophenol. Neither fenitro-oxon nor aminofenitrothion were found in any sample.

The limited available information indicates that neither fenitrothion nor the three degradation products were present in plants or invertebrates at concentrations likely to cause acute lethal effects on fish, birds or mammals that ingest them. However, further studies are needed to determine whether these residues could cause sublethal effects or affect aquatic invertebrates.

RECOMMENDATIONS

Since high concentrations of fenitrothion were present in Solenostoma sp. seven days after spraying, it is recommended that this bryophyte be sampled in the spring of 1982, before operational spraying, to determine whether residues have long-term persistence. In addition, future programs should sample other species of bryophytes and plants that do not die down in winter, and therefore could accumulate residues each year. The toxic effects on aquatic invertebrates of the ingestion of fenitrothion residues should be assessed.

1.1 INTRODUCTION

Recent studies have determined that the forest spray fenitrothion may persist in aquatic ecosystems long after spray application. Moody et al. (1978) reported low but stable concentrations of .12 to .15 µg/g fenitrothion in the vascular plant Ceratophyllum demersum 8 days after a stream was sprayed. Morrison and Wells (1981) reported similar low concentrations of fenitrothion in the stream moss Fontinalis antipyretica which, however, decreased greatly within 48 hours. Microcosm studies with lake plants have shown that fenitrothion residue in plants can be as great as 488 times that in the surrounding water (Weinberger et al. 1981) and that bioaccumulation can persist several weeks. Unpublished results cited in Miyamoto (1978) indicate that the concentration of fenitrothion in one alga was 181 times that in water. Miyamoto reported a half-life for fenitrothion in this alga of 2 days, but did not report the period during which residues were detected. However, Yule and Duffy (1972) found that, although there was a substantial decrease in the fenitrothion spray deposit on spruce and fir foliage in the first two weeks after spraying, in some cases about 10% (0.05 to 0.5 ppm) persisted throughout the following year. McNeil et al. (1979) found that post-spray fenitrothion residues in jack pine needles of 0.11 to 1.55 µg/g were associated with high larval mortality of jack pine sawfly.

Certain aquatic invertebrates consume algae, bryophytes and other aquatic plants, depending heavily on such material in the spring (Chapman and Demory, 1963) when forest spraying for spruce budworm usually occurs. Fenitrothion residues in aquatic plant tissue may have chronic or acute toxic effects on herbivorous stream invertebrates, waterfowl or mammals that feed on such plants. Several fenitrothion degradation products have been found at least as toxic as is the parent compound. These include fenitro-oxon (Miyamoto, 1978), 3 methyl-4-nitrophenol (Zitko and McLeese, 1980) and 3-methyl-4-aminophenol (Miyamoto, 1978), a degradation product of aminofentrothion. None of the previous fenitrothion studies has

determined the residues of fenitrothion, or its degradation products, in stream plants in New Brunswick. A survey was therefore conducted to determine the amount and degradation rate of fenitrothion and three degradation products in aquatic plants and herbivorous invertebrates from two experimentally-sprayed streams. This program was conducted in cooperation with Dr. D.C. Eidt (Maritime Forest Research Centre, Canadian Forestry Service) and Dr. V. N. Mallet (University of Moncton) who measured the spatial and temporal distribution of fenitrothion and selected degradation products in water and sediment, and assessed effects on invertebrate drift.

2.1 MATERIALS AND METHODS

Two study streams were used, Manzer Brook (46° 20'N, 67° 01'W) a tributary of the Nashwaak River in central New Brunswick and Otter Brook (45° 35.5'N, 66° 50'W), a tributary of the Northwest Oromocto River in southern New Brunswick (Plates 2-1 and 2-2). Manzer Brook is a cold, high gradient, headwater stream with dense conifer forest cover. Two sampling areas were selected on Otter Brook, which is a relatively low gradient stream with large ponds upstream from the experimental spray zone. Site 1, the upstream site, was in an open area with dense alder and willow shoreline vegetation. Site 2 had a deciduous forest canopy and dense shoreline bracken. All sampling sites had mainly a boulder, cobble and pebble substrate. Sampling site mean width, depth, velocity, discharge, distance from the spray zone, water chemistry and recorded temperature range during the sampling period are presented in Table 2-1. pH was measured on-site using a Fisher Accumet pH metre. Velocity measurements were taken at 0.5 m intervals along transects spaced 1.0 m apart in Manzer Brook, and at 1.0 m intervals with 2.0 m spacing in Otter Brook. Measurements were taken using an A.Ott Model C1 Flowmeter at 0.6 of the total depth, as an estimate of mean velocity (Hynes, 1970).

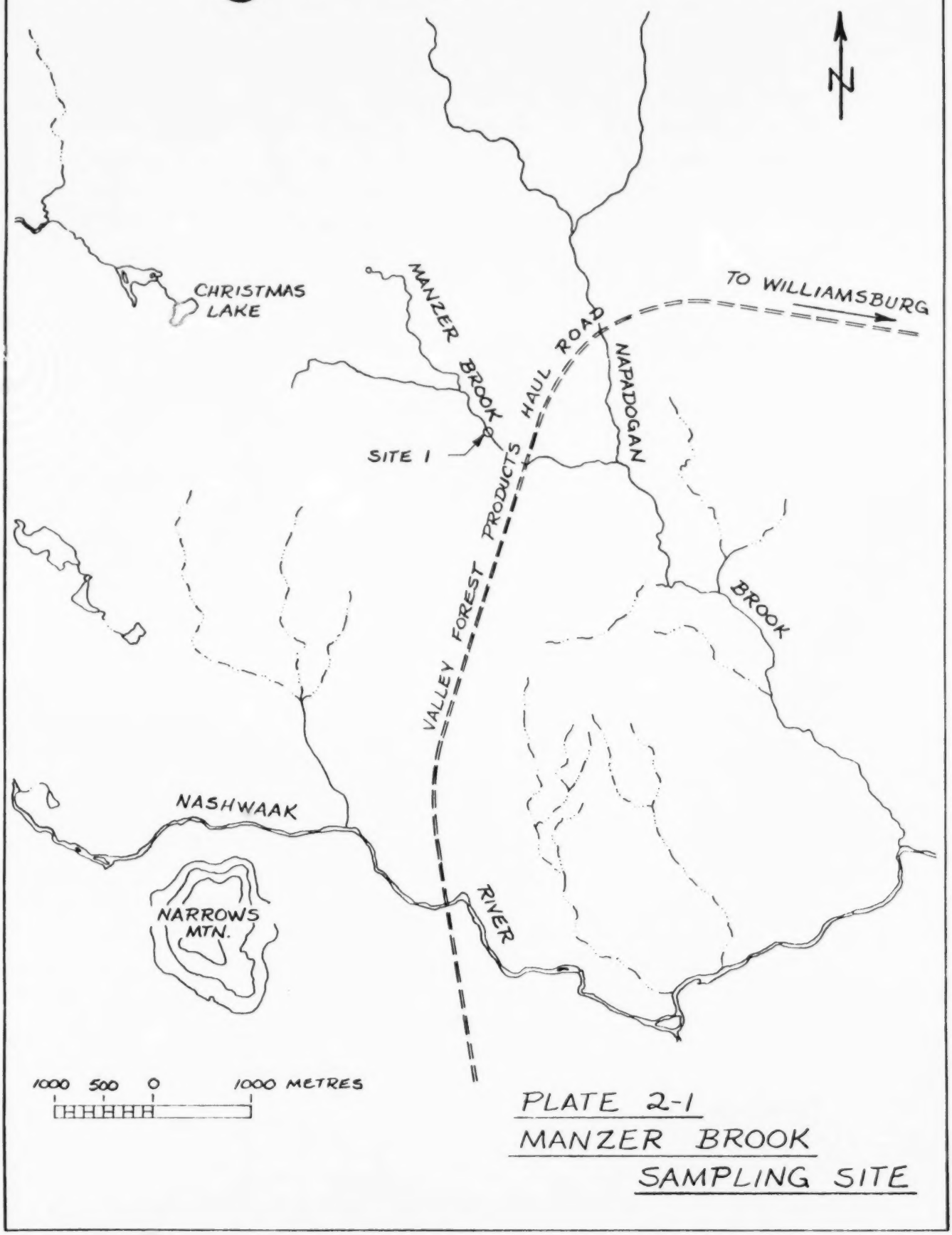

PLATE 2-1
MANZER BROOK
SAMPLING SITE

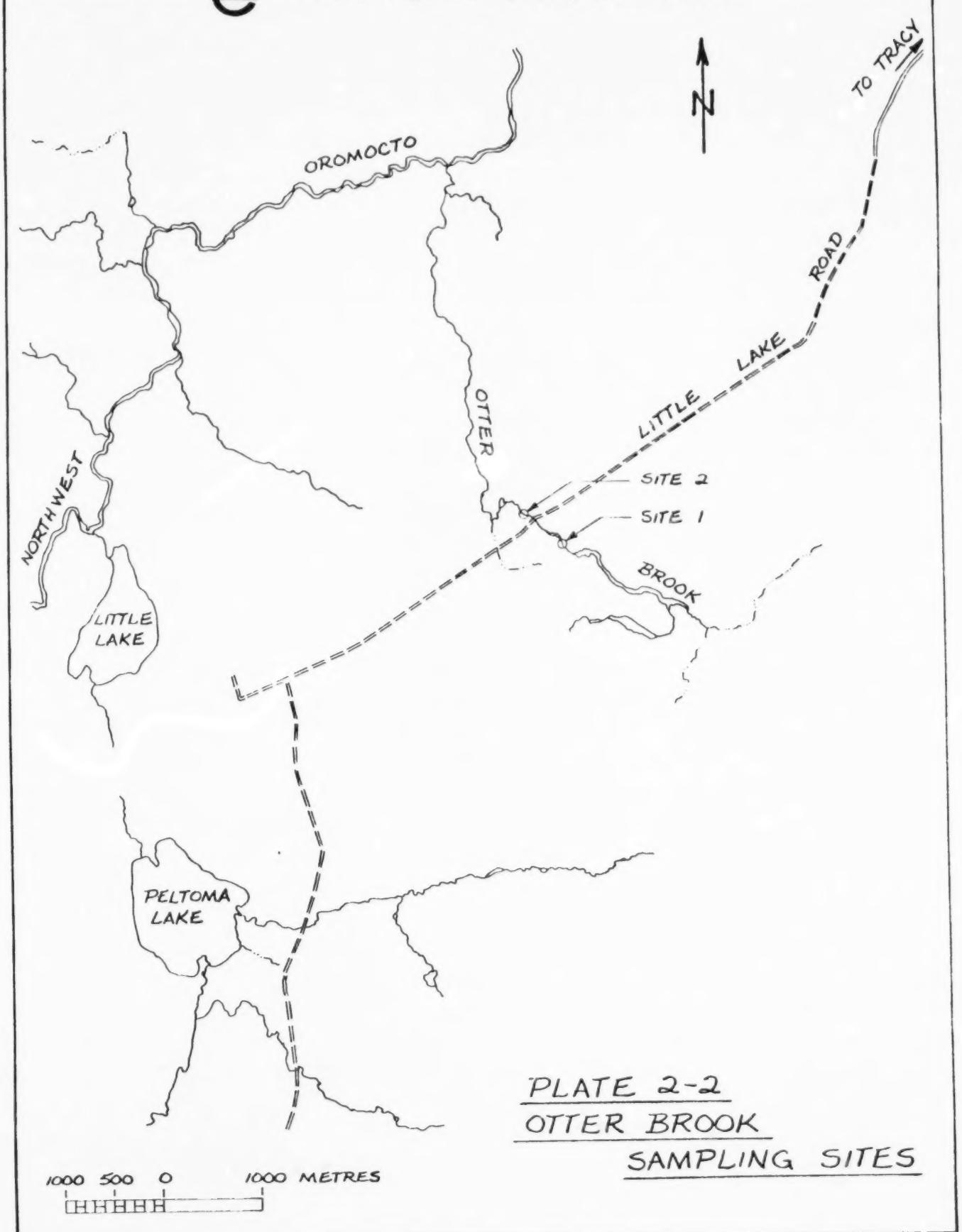

PLATE 2-2
OTTER BROOK
SAMPLING SITES

TABLE 2-1

Physical Measurements of the Sampling Sites

and Stream Water Chemistry

Parameters	Manzer Brook	Otter Brook Site 1	Otter Brook Site 2
Mean Site Width (m)	4.6	6.7	6.2
Mean Site Depth (cm) (range in parentheses)	24 (0-59)	22 (14-30)	18 (10-28)
Mean Velocity in Sampling Area (cm/s) (range in parentheses)	14.1 (0-36.7)	15.9 (0-40.0)	24.6 (7.6-38.9)
Distance from Spray Zone (m)	67	67	478
Discharge (L/s)	180	188	
Temperature (°C)	10-11	12-17	
Turbidity (JTU)	0.42	0.90	
pH	6.3-7.7	6.1-6.2	
Specific Conductance (μmhos/cm)	38	16	
Alkalinity (mg/L as $CaCO_3$)	11	6.2	
Hardness (mg/L as $CaCO_3$)	9.5	5.5	
Major Cations			
Calcium (mg/L)	3.0	1.5	
Magnesium (mg/L)	0.49	0.43	
Sodium (mg/L)	1.7	1.3	
Potassium (mg/L)	0.46	0.12	
Major Anions			
Sulphate (mg/L)	4.3	4.5	
Chloride (mg/L)	6.1	6.1	
Total Nitrogen (mg/L as N)	0.18	<0.01	
Reactive Silica (mg/L as SiO_2)	7.3	2.5	

Manzer Brook is located in operational spray block 105, but the Manzer watershed was not sprayed until June 4, after the completion of the sampling program. Spray lines about 3 km from the headwaters of Manzer Brook were sprayed on June 1, but there was no evidence in water samples (Table 2-2) of drift contamination of the brook. Otter Brook is about 10 km from the nearest operational spray block (92) which was sprayed on June 1. Both streams were sprayed during the 1980 spruce budworm control program.

On 26 May 1981, fenitrothion EC (95% technical fenitrothion containing 14.00 g active ingredient, Atlox 3409T, and ~~Aerotex 2470~~ Dowanol in water) was applied to a 52 m stretch of Manzer Brook, about 520 m upstream from the Valley Forest Products haul road. Spray was applied by Dr. D.C. Eidt, using a mistblower for an 83 minute period beginning at 9.42 (EST). The application rate was designed to achieve an instream fenitrothion concentration of 15.6 µg/L, which was thought sufficient to induce invertebrate drift. On June 9, fenitrothion in oil (97% technical fenitrothion containing 51.32 g active ingredient, and Cyclosol in 585 oil) was applied by mistblower to a 60 m stretch of Otter Brook about 400 m east of the road between Tracy and Mount Pleasant. Spray was applied over an 86 minute period beginning at 9:54 (EST), at a rate designed to achieve 52.3 µg/L. Spray formula components were supplied by Forest Protection Limited and were fresh stock from the 1981 spray program.

In Manzer Brook, the caddisfly Pycnopsyche sp., vascular plant water-mat (Chrysosplenium americanum), and red alga Batrachospermum sp. were collected in one shoreline pool, while the liverworts Riccia sp. and Solenostoma sp. and Heptageniid mayflies were collected throughout the sampling site. In Otter Brook, the vascular plants swamp candle (Lysimachia terrestris), slender spikerush (Eleocharis acicularis), manna grass (Glyceria borealis), the green alga Draparnaldia sp. and the aquatic moss Fontinalis sp. were collected at Site 1. Solenostoma sp., Simulium sp. and Neophylax sp. were collected at Site 2.

TABLE 2-2

Fenitrothion Content of Water Samples
Collected in Manzer and Otter Brooks

Stream	Prespray	6h Postspray	24h Postspray	96h Postspray	168h Postspray
Manzer Brook	NS	TR	TR	TR	TR
Otter Brook	0.02 *	0.03	0.07	TR	TR

Units: µg/L

TR traces <0.025 present
NS not sampled

* Rounded from 0.025 µg/l. See p. 9.

Plants and invertebrates, except for Simulium sp. were collected the day before spraying and at 6, 24, 96, and 168 hours after spraying. Pre-spray samples of Simulium sp. were collected on the morning of the spray day and were not collected at 168 hours after spraying because most had pupated in the sampling area, and adequate samples could not be obtained. Individuals of each species were collected by hand from various locations within each area in an effort to secure a representative sample. Only submerged organisms were collected. Plant roots and dead tissue were removed from samples, as were caddisfly cases and dipteran larvae in algal samples. Samples were rinsed with distilled water, drained, then placed in the preservative ethyl acetate in glass mason jars with aluminum foil seals. Samples were then transported to the laboratory on dry ice. One litre surface water samples were collected on each sampling trip, except the pre-spray trip to Manzer Brook, and preserved on XAD_2 resin columns for later analysis by the Research and Productivity Council.

All pesticide analyses were done by gas chromatograph at the Chemistry Department of the Research and Productivity Council in Fredericton, New Brunswick. Plant and animal samples were analyzed for fenitrothion, aminofenitrothion, fenitro-oxon and 3-methyl-4-nitrophenol. Analytical methods by Mr. D. Kirby of RPC are described in Appendix I. Since RPC stored certain extracts over a long period, they were required to determine the stability of preserved extracts spiked with either fenitrothion or one of the three degradation products investigated in this study and stored for 56 days. Of the four compounds tested only aminofenitrothion decreased considerably in concentration (Appendix II). Percent recovery of aminofenitrothion was 9, 29 and 54% for the three stored samples. Duplicates of whole plant and animal samples from Manzer Brook were divided and one half of each was sent to Dr. V. Mallet. Extracts from Otter Brook samples were sent for duplicate analyses.

3.1 RESULTS

Traces of fenitrothion (<0.025 µg/L) were found in water samples collected in Manzer Brook 6 hours after spraying and thereafter (Table 2-2). In Otter Brook, the pre-spray water sample contained 0.02 µg/L fenitrothion, which is at detection limit (Table 3-1) conservatively rounded to the next lower number. Twenty-four hours after spraying, a fenitrothion concentration of 0.07 µg/L was detected in Otter Brook. Thereafter, trace amounts were detected.

Measurable amounts of fenitrothion were detected in all organisms on at least one occasion (Tables 3-2 and 3-3). The liverwort Solenostoma sp. also contained pre-spray fenitrothion residues, as much as 0.48 µg/g (dry weight) in Otter Brook. Much lower pre-spray fenitrothion residues were also present in Solenostoma sp. collected in Manzer Brook and in swamp candle, manna grass and Draparnaldia sp. In addition, all invertebrates sampled had detectable, pre-spray fenitrothion concentrations, ranging from a low of 0.10 µg/g in Heptageniidae from Manzer Brook to a high of 2.22 µg/g in Simulium sp. from Otter Brook.

Organisms varied greatly in their apparent rates of fenitrothion uptake and degradation or excretion. Fontinalis sp., slender spikerush, and manna grass all had their highest measured fenitrothion residues 6 hours after spraying, and their fenitrothion content decreased steadily thereafter. However, the fenitrothion content of manna grass decreased at a slower rate than did that of the other two plants. Other plants had their highest measured residue not on the spray day but later in the sampling program. This was the case for Chrysosplenium, which peaked 4 days later and with Riccia sp., swamp candle and Draparnaldia sp., which peaked 24 hours after spraying. Solenostoma sp. had the highest plant fenitrothion residue found in this study, 9.0 µg/g measured 24 hours after the spraying of Otter Brook. However, delayed uptake did not appear to occur in Solenostoma sp. collected in Manzer Brook. Furthermore, the fenitrothion

TABLE 3-1

Detection Limits and Average Percent Recovery of Spiked Samples

	Recovery	Detection Limit
fenitrothion	100%	0.025
3-methyl-4-nitrophenol	85%	0.025
fenitro-oxon	67%	0.1
aminofenitrothion	40%	0.05

Units: µg/ml of solution injected into the gas chromatograph.

TABLE 3-2

Concentrations of Fenitrothion and Degradation Products in Aquatic Plant and Animal Tissues Sampled in Manzer Brook. (Duplicate Analyses by V. Mallet in Parentheses)

Organism	Compound	Prespray	6h Postspray	24h Postspray	96h Postspray	168h Postspray
Solenostoma sp.	fenitrothion	0.09	2.94	1.17(2.46)	2.31	0.95
	3-methyl-4-nitrophenol	ND	ND	ND	ND	ND
water-mat *Chrysosplenium americanum*	fenitrothion	ND	0.41	0.52	0.64	0.13
	3-methyl-4-nitrophenol	ND	ND	ND	ND	ND
Batracho-spermum sp.	fenitrothion	ND	ND	0.33(ND)	0.17	-
	3-methyl-4-nitrophenol	ND	ND	ND	ND	
Riccia sp.	fenitrothion	ND	0.03	0.49	0.23	-
	3-methyl-4-nitrophenol	ND	ND	ND	ND	
Heptageniidae (mainly *Epeorus*)	fenitrothion	0.10	ND	4.10	1.19	ND
	3-methyl-4-nitrophenol	ND	ND	0.51	ND	ND
Pycnopsyche sp.	fenitrothion	0.30	0.15(ND)	1.30	0.58	ND
	3-methyl-4-nitrophenol	ND	ND	ND	ND	ND

Units: µg/g dry weight
ND nondetectable
hyphen no analysis

TABLE 3-3

Concentrations of Fenitrothion and Degradation Products in Aquatic Plant and Animal Tissues sampled in Otter Brook. (Duplicate Analyses by V. Mallet in Parentheses)

Organism	Compound	Prespray	6h Postspray	24h Postspray	96h Postspray	168h Postspray
Solenostoma sp.	fenitrothion	0.48	4.96(3.25)	9.0	1.49	2.91
	3-methyl-4-nitrophenol	0.12	0.09	ND	ND	ND
Fontinalis sp.	fenitrothion	ND	0.68(0.95)	0.12(0.19)	0.08	0.01
	3-methyl-4-nitrophenol	ND	ND	ND	ND	ND
swamp candle (Lysimachia terrestris)	fenitrothion	0.06	0.08	1.00	0.30	0.08
	3-methyl-4-nitrophenol	ND	ND	ND	ND	ND
slender spikerush (Eleocharis acicularis)	fenitrothion	ND	0.63	0.07	0.08	ND
	3-methyl-4-nitrophenol	ND	ND	ND	ND	ND
manna grass (Glyceria borealis)	fenitrothion	0.02	0.97	0.10	0.08	0.07
	3-methyl-4-nitrophenol	ND	0.06	ND	ND	ND
Draparnaldia sp.	fenitrothion	0.02	0.03	0.73	0.05	ND
	3-methyl-4-nitrophenol	ND	ND	ND	ND	ND
Simulium sp.	fenitrothion	2.22	2.21	10.0	4.08	-
	3-methyl-4-nitrophenol	ND	ND	ND	ND	
Neophylax sp .	fenitrothion	0.24	0.65	5.36	0.98	0.38
	3-methyl-4-nitrophenol	ND	ND	ND	ND	ND

content of Solenostoma sp. samples did not decrease steadily with time as was the case for most other organisms and 2.91 µg/g was detected in a sample collected in Otter Brook 7 days after spraying.

As with some of the plant samples, there was a slow rate of fenitrothion uptake by all sampled invertebrates and the highest residues were found 24 hours after spraying. Simulium sp. with 10.0 µg/g, had the highest fenitrothion concentration of any invertebrate sampled. Concentrations in all invertebrates appeared to decrease thereafter; in Manzer Brook they had dropped below detection limits after 7 days.

Duplicate analyses by Dr. Mallet for fenitrothion of extracts from Otter Brook agree reasonably well with the results reported by RPC (Table 3-3), considering that their analytical methods differed (Appendix III). However, duplicate analyses on whole plant and invertebrate samples from Manzer Brook (Table 3-2) differed to a greater extent.

Low levels of 3-methyl-4-nitrophenol were detected in Solenostoma sp. and manna grass 6 hours after spraying but not thereafter. A concentration of 0.12 µg/g was also found in the pre-spray Solenostoma sp. sample from Otter Brook. The greatest amount of 3-methyl-4-nitrophenol detected was 0.51 µg/g found in Heptageniidae collected 24 hours post-spray, but it was not detected in Heptageniidae collected before or after this sampling time. Dr. Mallet was unable to analyze for 3-methyl-4-nitrophenol, apparently due to equipment malfunctions. However, neither fenitro-oxon nor amino-fenitrothion was detected in either plant or invertebrate samples by RPC or Dr. Mallet.

4.1 DISCUSSION

The mistblower spraying of Otter and Manzer Brook simulated operational forest spraying quite well. The highest fenitrothion concentrations

detected in water at the Manzer and Otter Brook plant and invertebrate sampling sites were 4.1 and 11.3 µg/L respectively (Eidt, 1981). In both streams, fenitrothion concentrations had decreased to less than 1% of the peak concentration after 24 hours. Symons (1977) surveyed the literature on the effects of forest spraying and found that peak fenitrothion concentrations in streams after operational spraying ranged from 1.33 to 57 µg/L (median 4.8 µg/L). In most cases where data were available, concentrations had decreased to 10% of the peak after 24 hours.

Fenitrothion was detected in the pre-spray water sample collected in Otter Brook and could have resulted either from drift or from accidental spraying of the watershed. Since the pre-spray sample from that brook was collected on June 8, an accidental spray release from a Forest Protection Limited spray plane that occurred near the Otter Brook watershed the morning of June 9 could not be the source (Forest Protection Limited, 1981). Another potential, although improbable, source of contamination may have been spraying near Juvenile Settlement, about 15 km to the east of May 22.

Previous forest spray studies by Montreal Engineering Company, Limited (1977, 1979) reported traces and quantifiable concentrations of pesticide in stream water collected as much as 50 km from the nearest spray block. Pearce et al. (1980) reported a mean fenitrothion concentration of 0.34 µg/L in 17 contaminated rain samples out of a total of 33 samples collected outside spray blocks. It is impossible to trace the exact source of the pre-spray contamination of Otter Brook, and it appears difficult to prevent such contamination in New Brunswick forest spray studies.

It is probable that the pre-spray fenitrothion residues found in plants and invertebrates in Otter Brook were at least in part a result of pre-spray pesticide drift or other contamination. The unusually high pre-spray residues in Solenostoma sp. and Simulium sp. suggest that these two organisms may be useful indicators of fenitrothion contamination, even after

residues in water dropped below detection limits. Lakshiminarayana (1980) analyzed lake phytoplankton and algae after operational spraying as potential indicators of contamination, and found residues ranging from trace levels to 0.05 mg/L (µg/g) just after spraying. In the present study, higher pre-spray residues were found in Solenostoma sp. swamp candle, and all four invertebrate species than in either of the algae (Draparnaldia sp., Batrachospermum sp.) that were sampled. Pre-spray fenitrothion residues of 0.15 µg/g were also found by Penney (1971) in mayfly nymphs, apparently in the absence of quantifiable concentrations in water.

The bioaccumulation ratios (concentration of fenitrothion in organism (dry weight)/concentration in water) for Solenostoma sp. in Manzer and Otter Brooks were 695 and 769 respectively at peak concentration. Pre-spray residues in water and plants, where measured, were subtracted from peak values recorded for each. The bioaccumulation ratios for Solenostoma sp. appear to be higher than any previously reported for aquatic plants. Moody et al. (1978) reported 4.19 ppm fenitrothion in duckweed (maximum subsurface water concentration of 9.46 ppb) but suggested this was due to direct deposition on the floating duckweed. Weinberger et al. (1981) reported bioaccumulation ratios of 370-488 for the plants Myriophyllium, Sagittaria and Elodia in microcosm studies and 500 and 230 for Chlamydomonas and Chlorella in field models. Their spray application rates in field and microcosm studies were designed to attain either 1 or 10 ppm in water. Morrison and Wells (1981) found fenitrothion residues of, at most, 0.139 mg/kg (wet weight) in willow moss (Fontinalis antipyretica) exposed to a maximum of 18 µg/L in water. Fenitrothion residues of Fontinalis sp. sampled in Otter Brook were similar to the results (wet weight) reported by Morrison and Wells if the Otter Brook results (dry weight) are adjusted to allow for an 80% moisture content (Appendix IV).

Two types of fenitrothion accumulation by plants are suggested by these results. Direct and immediate absorption or adsorption from water occurred in Fontinalis sp., manna grass and slender spikerush. All of these plants were collected at Site 1 on Otter Brook, which was open and exposed to direct light. Weinberger et al. (1981) reported that light enhanced the rate of fenitrothion uptake by aquatic plants.

Swamp candle, Riccia sp. and Chrysosplenium all increased their fenitrothion content between 6 and 24 hours after the spraying, when the fenitrothion content of the water was less than 1% of the peak. This suggests fenitrothion uptake from contaminated sediment either on foliage or, for vascular plants, through roots. Moody et al. (1977) demonstrated the translocation of labelled fenitrothion in conifers.

Solenostoma sp. fenitrothion residues did not decrease steadily with time as did most other samples. Dr. Mallet's duplicate analysis on whole Solenostoma sp. from Manzer Brook differed greatly from that done by RPC. This suggests that individual clusters of Solenostoma sp. differed in their fenitrothion content, perhaps due to different locations in the stream, thus influencing results accordingly.

Symons (1977) summarized literature on the acute effects on birds and mammals of ingesting fenitrothion. All LD_{50} values that he reported were much higher than the values reported here. For birds, 157 ppm in dry food fed to bobwhite (Colinus virginianus) over a five day period was the lowest LD_{50} reported based on concentration in food. Miyamoto (1977) found no sublethal effects on mallard ducks and white rats due to the long-term ingestion of 100 mg/kg fenitrothion in food. However, Busby (1981) found that single doses of fenitrothion as low as 7 mg/kg (body weight) caused growth abnormalities in white-throated sparrow nestlings. The available literature suggests that the plant residues detected in this study are unlikely to cause acute lethal effects on mammals or birds that may feed on them. However, further studies with relevant aquatic birds and mammals are needed to determine if sublethal effects could occur.

McNeil et al. (1979) reported that fenitrothion residues of 0.11 to 0.55 ppm in jack pine foliage were associated with high rates of mortality in jack pine sawfly, which fed on jack pine. There are apparently no ingestion studies on aquatic invertebrates to use in assessing risk associated with the ingestion of the fenitrothion concentrations reported here.

The peak fenitrothion concentrations detected in Simulium sp., Neophylax sp., and Heptageniidae were higher than the 3.19 ppm that Penney (1971) reported for mayfly nymphs exposed to a maximum of 0.057 ppm fenitrothion in water. Comparisons are, however, difficult because Penney did not state whether residues in insects were based on wet weight or dry weight, nor did he identify to family of mayflies sampled.

Leonhard (1974) reported the uptake of fenitrothion by crayfish after detectable amounts had disappeared from stream water and sediment. In the present study, four invertebrate species showed fenitrothion uptake between 6 and 24 hours after spraying, when measured concentrations had dropped to less than 1% of the peak concentration. The source of this fenitrothion cannot be determined from the available information, but ingestion or contact with contaminated plants or sediment are possible sources. Epeorus, Neophylax and Pycnopsyche (in the last instar) all scrape algae and detritus from rocks, and Simulium filter fine particulate organic matter from the water (Merritt and Cummins, 1978). Chapman and Demory (1963) found that another caddisfly Micrasema feeds mainly on moss and liverwort.

In samples from both streams, Eidt (1981) found a positive correlation between the fenitrothion content of sediment and its organic content. He also found that nearly 50% of the fenitrothion in water samples was on suspended sediment. Through feeding on suspended organic sediment, Simulium may acquire high fenitrothion residues. Fredeen (1962) reported that blackfly larvae ingest more DDT in turbid water than in clear water.

The fenitrothion residues detected in invertebrates in this study do not appear to be potentially toxic to fish. Wildish and Lister (1973) reported that no behavioural or physiological effects were found when brook trout (Salvelinus fontinalis) ingested 1,000 ppm fenitrothion in food (wet weight) daily for a four week period. However, the aquatic insects Plecoptera, Trichoptera and Coleoptera all have dominant taxa that consume aquatic insects (Merritt and Cummins, 1978) and may be at risk. Potential effects on these taxa cannot be predicted from the available literature.

A fenitrothion degradation product was only found in one invertebrate sample, the 24 hour post-spray Heptageniidae sample, which contained 0.51 µg/g 3-methyl-4-nitrophenol, a hydrolysis product of fenitrothion (Miyamoto, 1978). Penney (1971) reported that no degradation products were found in post-spray mayfly samples, but did not specify the products for which he analyzed. Low concentrations of 3-methyl-4-nitrophenol were also found in three plant samples collected in Otter Brook. Since this compound was not detected later in the sampling program, as fenitrothion residues decreased, it does not appear to be a major degradation product in these plants. However, Weinberger et al. (1981) found an increase in the concentration of 3-methyl-4-nitrophenol in Elodea and Sagittaria over time. They also reported substantial amounts of fenitro-oxon in Myriophyllum. These results may have been due to a higher spray application rate designed to achieve 10 ppm, a rate many times greater than that used in the present study.

Some of the following processes could account for the decrease in fenitrothion concentration in plant and invertebrate samples:

1) other routes of degradation (Miyamoto, 1977) may be followed than those involving the three compounds for which analyses were performed

2) compounds along the degradation route may be briefly present at concentrations less than detection limits of the methods used in this study

3) in invertebrates, fenitrothion may be excreted without degradation

4) the principal degradation products, on formation, may bind with other compounds to form stable complexes that are not extractable, as Lockhart et al. (1977) and Maguire and Hale (1980) have suggested is the case for aminofenitrothion.

5) fenitrothion or its degradation products may be translocated to roots or other plant parts not sampled in this study.

Aminofenitrothion was the only degradation product to decrease substantially in concentration in preserved, spiked extracts (Appendix II). Whether this resulted from binding or degradation is not known. Improved analytical techniques may be needed to determine if binding occurred.

ACKNOWLEDGEMENTS

We thank Dr. G.B. Bacon (Research and Productivity Council), Dr. D.C. Eidt, Dr. H.R. Hinds (University of New Brunswick) and Dr. H. van Groenewoud (Maritimes Forest Research Centre) for the identification of algae, Neophylax sp. and Epcorus sp., vascular plants and bryophytes respectively. We also thank Dr. V.C. Mallet for duplicate plant sample analyses and Environment New Brunswick for water sample analyses.

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Appendix I

Analytical Procedures

The following analytical procedures and comments were reported by Mr. D. Kirby, Chemistry Department, New Brunswick Research & Productivity Council.

1. All biota samples were received immersed in ethyl acetate and were stored at $4^{o}C$ prior to analysis.

   Each sample was macerated in its preservative, vacuum filtered and diluted to 100 ml with ethyl acetate, on occasions after concentration of the solvent under vacuum (Buchi Roto-vap).

   Initial work involved sample clean-up and an attempt to separate the four compounds of interest on alumina columns. This revealed that neither aminofenitrothion nor fenitro-oxon could be recovered from the alumina column and a change was made to 2 g silica gel (E Merck Silica Gel 60, Cat. #7734, deactivated with 3% H_2O) for all samples.

   Three eluate fractions (20 ml each) were collected from the silica gel columns:

   Fraction I: containing fenitrothion and 3-methyl-4-nitrophenol in 10% ethyl acetate in hexane

   Fraction II: containing fenitro-oxon in 25% ethyl acetate in toluene

   Fraction III: containing aminofenitrothion in 5% diethylamine in toluene

   Each fraction was concentrated to 1 ml and analysed using a Varian 3700 gas chromatograph equipped with a 6' x 1/4" 3% OV-17 on Chromosorb W-HP (80/100 mesh) column and a Thermionic Specific Detector (TSD).

   Spikes and Detection Limits

   Biota spikes were prepared, at the 1 µg level, using grass and were inserted into the routine procedure at intervals.

   Solids Content

   The entire "wet" mass of biota material from the filters was weighed and a sub-sample taken for determination of its total solids content.

2. Water Samples

"Water samples" were, in fact, columns containing XAD-2 resin. The resins were extracted by soaking in 20 ml toluene followed by a 5 ml toluene wash*. The combined extracts were dried, evaporated to 1 ml and analysed as previously described.

Spikes and Detection Limit

XAD-2 resin was spiked with 1 µg fenitrothion. Recovery was 79.5% and the detection limit was 0.025 µg/ml of injected solution.

Discussion

Neither fenitro-oxon nor aminofenitrothion were detected in any of the submitted biota samples. In those few cases where peaks of similar retention times to those of the standards were obtained, the fractions were further concentrated and aliquots analysed by GC/MS using a Finnigan 4021 with INCOS data system which confirmed the absence of these metabolites.

* "Fenitrothion Sprayed on a Pond: Kinetics of Its Distribution and Transformation in Water and Sediment, J. Agric. Food Chem., 1980, 28, 372-378".

Appendix II

Percent Recovery of Spiked Extracts

From Otter Brook after a Storage Period of 56 Days

Extract	fenitrothion µg/1 mL	3-methyl-4-nitrophenol µg/1 mL	fenitro-oxon µg/1 mL	amino-fenitrothion µg/1 mL
Solenostoma sp. (Prespray)				
Total (µg)	1.18	1.15	1.27	0.09
Added (µg)	1.0	1.20	1.20	1.0
Aliquot of Sample (µg)	0.33	0	0	0
Recovery (%)	85	96	106	9
Neophylax sp. (Prespray)				
Total (µg)	0.98	1.20	1.06	0.54
Added (µg)	1.0	1.20	1.20	1.0
Aliquot of Sample (µg)	0.01	0	0	0
Recovery (%)	97	100	88	54
Draparnaldia sp. (96 h postspray)				
Total (µg)	1.23	1.45	1.40	0.29
Added (µg)	1.0	1.20	1.20	1.0
Aliquot of Sample (µg)	0.07	0	0	0
Recovery (%)	116	121	117	29

Appendix III

Analytical Procedures Used By

Dr. V. Mallet

METHODS

1. Processing of XAD Columns

Each column was eluted with 3 x 30 mL of ethyl acetate and the eluate was concentrated to 1 mL for GC analysis.

2. Processing of Biota Extracts

The whole extract was evaporated almost to dryness, dissolved in 50 mL of acetonitrile, diluted with 2L of water and processed as with biota samples.

3. Processing of Biota Samples

The whole sample was blended for five minutes with 150 mL of acetonitrile, filtered through a Buchner funnel. The filtrate was diluted with 2 L distilled water and the whole solution was passed through an XAD-7 column. The column was washed with 1 L of distilled water and then eluted with 3 x 30 mL of ethyl acetate. The eluate was concentrated to 1 mL (for GC analysis).

4. Determination of Nitrocresol

Analysis for this derivative was performed on over 20 samples overall, by HPLC. None was ever detected but since there was a lot of interfering co-extractive due to the XAD-2 resin any definite conclusion would be hasty.

Appendix IV

Moisture Content of Tissues Collected in Otter Brook *

Organism	Weight of Wet Samples (g)	Percent Moisture
Solenostoma sp.	2.709	83
Fontinalis sp.	2.807	80
swamp candle (Lysimachia terrestris)	4.376	92
slender spikerush (Eleocharis acicularis)	2.197	89
manna grass (Glyceria borealis)	3.515	89
Draparnaldia sp.	12.917	97
Neophylax sp.	1.018	80
Simulium sp.	0.758	93

* Samples were dried for 18 h at 110° C in an air-circulating oven.

ADDENDUM TO THE NOVEMBER 1981 REPORT TO ENVIRONMENT NEW BRUNSWICK ENTLITLED "FENITROTHION ACCUMULATION BY PLANTS AND INVERTEBRATES IN TWO EXPERIMENTALLY SPRAYED STREAMS IN NEW BRUNSWICK"

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ENVIRONMENT N. B.
ENVIRONMENTAL SERVICES BR.
AUG 25 1982
FILE.....
X FILE.....

9 August 1982

Dr. D. Besner
Environmental Services Branch
Environment New Brunswick
P.O. Box 6000
Fredericton, New Brunswick
E3B 5H1

Dear Dave:

Enclosed is the report on the monitoring efforts which were aimed at determining whether fenitrothion persists in aquatic plants and sediments for a full year. As you will see from the results, fenitrothion persisted in liverwort tissues, but not in sediments. As previously discussed, the presence of fenitrothion in liverworts was confirmed by analyses performed by Dr. Mallet. Those results will be presented with the results of the aminocarb project.

Thank you for the opportunity to complete this study.

Yours very truly,

G. Michael DeGraeve
Senior Biologist

GMD/cet
cc: Mr. K.D. Phinney
Mr. A.J. Sosiak

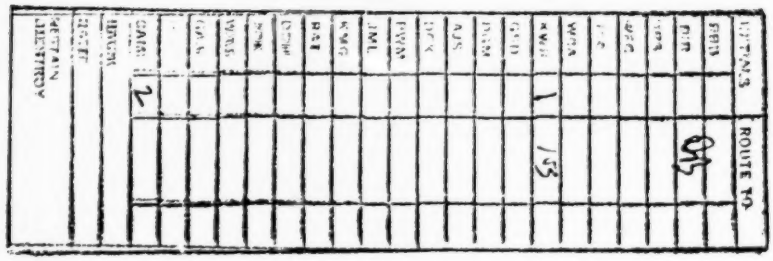

TABLE 1-1

Concentrations (µg/g dry weight) of Fenitrothion and 3-methyl-4-nitrophenol in liverwort and sediment samples from Manzer Brook[a] and Otter Brook[b]

Manzer Brook			Otter Brook		
Substrate	Compound	Concentration	Substrate	Compound	Concentration
Sediment Sample 1	Fenitrothion	N/D	Sediment Sample 1	Fenitrothion	N/D
	3-methyl-4-nitrophenol	N/D		3-methyl-4-nitrophenol	N/D
Sediment Sample 2	Fenitrothion	N/D	Sediment Sample 2	Fenitrothion	N/D
	3-methyl-4-nitrophenol	N/D		3-methyl-4-nitrophenol	N/D
Riccia sp.	Fenitrothion	0.6	*Solenostoma* sp. Sample 1	Fenitrotthion	1.6
	3-methyl-4-nitrophenol	N/D		3-methyl-4-nitrophenol	N/D
Solenostoma sp.	Fenitrothion	3.6	*Solenostoma* sp. Sample 2	Fenitrothion	0.9
	3-methyl-4-nitrophenol	N/D		3-methyl-4-nitrophenol	N/D
			Solenostoma sp. Sample 3	Fenitrothion	1.7
				3-methyl-4-nitrophenol	N/D

[a] Sediment samples and *Riccia* sp. collected on 19 May 1982; *Solenostoma* sp. sample collected 1 June 1982

[b] Sediment samples collected on 24 May 1982; *Solenostoma* sp. samples collected 24-26 May 1982

In 1981 Montreal Engineering Company, Limited monitored fenitrothion and 3-methyl-4-nitrophenol in both plant and benthic invertebrate tissues in Manzer Brook and Otter Brook following an experimental application of fenitrothion to the streams. Trace amounts (<0.025 µg/L) of fenitrothion were found in water samples from Manzer Brook up to 168 hours after the experimental application, and the concentrations in water samples from Otter Brook ranged from 0.07 µg/L 24 hours postspray to trace levels 96 and 169 hours postspray (Sosiak, 1981). Eidt (1982) also monitored fenitrothion levels in organic sediments following the same experimental spray application.

Both fenitrothion and 3-methyl-4-nitrophenol were found in plant and benthic invertebrate tissues and fenirtothion was detected in organic sediments. Fenitrothion was particularly persistent in tissues of the liverwort Solenostoma sp. Consequently, in late May/ early June of 1982 we collected liverwort and organic sediment samples to determine if fenitrothion and/or 3-methyl-4-nitrophenol were still present a full year after application of the insecticide.

Table 1 presents the results of the 1982 monitoring program. No fenitrothion or 3-methyl-4-nitrophenol was detected in organic sediments from Manzer or Otter Brooks. However, the liverworts Riccia sp. and Solenestoma sp. both contained detectable levels of fenitrothion about one year after the experimental applications, in some cases, more fenitrothion than was measured 7 days after spraying (Table 2). Although the sample size was small in both 1981 and 1982, the results indicate that Solenostoma sp. is a very good species for monitoring fenitrothion persistance in stream communities and that fenitrothion can persist in the tissues of this liverwort for at least one year. Because Solenostoma sp. is frequently abundant in New Brunswick streams, this species may be ideal to use as a tool for determining the level of long-term persistance of fenitrothion in areas repeatedly sprayed to control spruce budworm.

Report Number: C/82/72 July 23, 1982

Job Number: 6403/1-3

Client: Mr. M. DeGraeve,
Monenco Limited,
500 Beaverbrook Court,
Fredericton, New Brunswick

ANALYSIS OF VEGETATION AND SEDIMENT SAMPLES FOR FENITROTHION SAMPLES

RPC Codes:
6403/1: 82/208 to 82/210
6403/2: 82/218 to 82/222
6403/3: 82/347

On May 20 and 26, and June 11, 1982 a total of five (5) vegetation samples and four (4) sediment samples were received at RPC for analysis of their fenitrothion and 3-methyl-4-nitrophenol contents.

The samples, which contained ethyl acetate were stored at 5°C prior to analysis.

Vegetation

The samples were homogenised in situ, filtered and one quarter of the filtrate concentrated prior to clean-up on a charcoal/celite column. The final column eluate was concentrated to known volume and analysed by gas chromatography.

Sediments

(a) The supernatent ethyl acetate was decanted from each sample, the volumes noted, and then concentrated to known volume prior to GC analysis.

(b) Known weights of the "wet" sediments were mixed with three times their weight of anhydrous sodium sulphate and extracted in a Soxhlet appartus with ethyl acetate. The extracts were concentrated to known volume and analysed by GC.

.../2

TABLE 1-2

Comparison of 1981[a] and 1982 Fenitrothion Levels (µg/g)
in Plant Tissues in Manzer and Otter Brook

Manzer Brook			Otter Brook		
Riccia sp.	1981	0.23	*Solenostoma* sp.	1981	2.91
	1982	0.60		1982	1.60
					0.90
					1.70
Solenostoma sp.	1981	0.95			
	1982	3.60			

[a] Levels shown for 1981 are those detected 96 and 168 hours postspray for *Riccia* sp. and *Solenostoma* sp. respectively.

Report Number: C/82/72 July 23, 1982

RESULTS (Dry weight basis)

Job No.	RPC Code	Bottle Label	Dry Weight* (g)	Fenitrothion (µg/g)	3-Methyl-4-Nitrophenol (µg/g)
		(a) Vegetation			
6403/1	82/208	Manzer Brook, May 19, 1982. Liverworts. Riccia	2.92	0.6	N/D
6403/2	82/220	Otter Brook, May 24, 1982. Liverwort Sample A	10.3	1.6	N/D
6403/2	82/221	Otter Brook, May 24, 1982. Liverwort Sample B	8.3	0.9	N/D
6403/2	82/222	Otter Brook, May 26, 1982. Liverwort, Solenestoma	8.3	1.7	N/D
6403/3	82/347	Manzer Brook, June 1, 1982. Liverwort Sample	1.1	3.6	N/D
		(b) Sediments			
6403/1	82/209	Manzer Brook, May 19, 1982. Sediment Sample A	23.5 Supernatent	N/D N/D	N/D N/D
6403/1	82/210	Manzer Brook, May 19, 1982. Sediment Sample B	22.5 Supernatent	N/D N/D	N/D N/D
6403/2	82/218	Otter Brook, May 24, 1982. Sediment Sample A	2.3 Supernatent	N/D N/D	N/D N/D
6403/2	82/219	Otter Brook, May 24, 1982 Sediment Sample B	1.8 Supernatent	N/D N/D	N/D N/D

Note: *Dry weight for vegetation is the Total Dry Weight of material submitted and extracted. Dry weight for sediments is that contained in the portion of the sample taken for analysis.
N/D: less than lower limit of detection.

Cindy Henderson, Lab. Technician, Chemistry Department.

Steve Feiner, Trace Organics Lab. Supervisor, Chemistry Department.

Dr. Peter J. Silk, Head, Chemistry Department.

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Information emanating from this Council is given after the exercise of all possible care in its compilation, preparation and issue but is provided without liability in its application and use.

Analysis was conducted on a Varian Model 3700 gas chromatograph equipped with a Thermionic Specific Detector and fitted with a 6 ft. x 2 mm 3% OV-1 on Gas Chrom Q 100/120 mesh column.

The results of the analyses were confirmed on a Hewlett-Packard model 5840 gas chromatograph equipped with a nitrogen-phosporous detector and fitted with a 3% OV-101/6% OV-210 on Chrom W HP 80/100 mesh column.

Identification of fenitrothion using both gas chromatographic columns was required for positive confirmation of fenitrothion residues in the samples.

The lower limit of detection was 10ng/g for a 50 g (wet) sample of sediment.

.../3

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